ANNEXURES 1

Rama medical college hospital and research centre

Mandhana Kanpur

Consent form

I .....son/daughter/wife of.....exercising my free power of choice, hereby give my consent to be included in the study "SCREENING AND MOLECULAR CHARACTERIZATION OF VRSA AND VISA AMONG THE MRSA ISOLATES FROM THE PURIFIED COMPOUNDS OF CANNABIS SATIVA L. AND ALLIUM SATIVUM L."I hereby give my consent for information related to me to be reported in the research study.

I understand that my name and identity will be concealed.

Once signed, I cannot revoke my consent.

(To be explain in detail in native language)

सहमतिफार्म

मैं /पुत्र .....पुत्री/पत्नी/ .....स्वयंइसअध्ययनमेंभागलेनारहा/रहींहूं I

मैंस्वयंसेसंबंधीसूचनाइसअध्ययनकार्यमेंप्रकाशितकरनेकीसहमतिदेता/देतीहूं I

मैंसमझता/समझतीहूंकिमेरापहचानगोपनीयरखीजाएगी।

एकबारहस्ताक्षरकेपश्चातमैंसहमतिरद्दनहींकरसकता/ सकतीहूं I

सहभागी

नाम:.....

हस्ताक्षर/अगुठेकानिशान:.....

दिनाक:.....

ANNEXURES 2

PATIENT PROFORMA

CLINICAL PROFILE OF THE PATIENT:

Name of patient: …….. Sex : …… Age: ……

I.D.No:……..Address:……..

MICROBIOLOGICAL PROFILE OF THE PATIENT

DAY 1

Date and Time of sample collection:……..Clinical Specimen:……

Macroscopic Observation: Color: ……..... Appearance: ……

pH…… Others: ……..

Direct Microscopic Observation:

Direct Gram Staining / Wet mount (For urine):.....

Microscopic observation: …… Gram Staining: …….. Colour: ……..

Appearance:……..Others:……

Haemolysis:……AST:……TypeIsolated:……

Culture of specimen on: (a)Nutrient agar (5-7% NaCl) (b) Cled agar (c) Blood Agar

Incubation temperature and period…….

DAY 2: Reading Of Culture Plates

Media used	Shape	Size	Color	Texture	Haemolysis on BA	Lactose fermentation	Growth	
MacConkey Agar								
Blood Agar								
CLED Agar								

Gram-staining test: ……

Catalase test:..……..

Mannitol salt agar: ……..

Coagulase test: ……..

Dnase: .....

Bacitracin: .....

Optochin: .....

Blood agar plate: .....

Provisional Identification of Organism: ……

Biochemical Test and Identification of Staphylococcus aureus

Characteristics	*Staphylococcus aureus*
Gram Staining	Positive
Shape (Cocci/Diplococci/Rods)	Cocci
Motility (Motile / Non-Motile)	Non-Motile
Capsule (Capsulated/Non-Capsulated)	–
Spore (Sporing/Non-Sporing)	Non-Sporing
Flagella (Flagellated/Non-Flagellated)	–
Catalase	Positive (+ve)
MR	Positive (+ve)
VP	Positive (+ve)
OF (Oxidative/Fermentative)	Fermentative
Citrate	Positive (+ve)
Urease	Variable, Mostly Positive (+ve)
Nitrate Reduction	Positive (+ve)
Gelatin Hydrolysis	Positive (+ve)
Coagulase	Positive (+ve)
Hemolysis (Alfa/Beta/Gamma)	Beta Hemolysis

Pigment	Positive (+ve)
Blood agar	Positive(+ve)
Fermentation Of	
DNase	Positive (+ve)
Fructose	Positive (+ve)
Glucose	Positive (+ve)
Lactose	Positive (+ve)
Maltose	Positive (+ve)
Mannitol	Positive (+ve)
Mannose	Positive (+ve)
Sucrose	Positive (+ve)
Enzymatic Reactions	
Acetoin Production	Positive (+ve)
Alkaline Phosphatase	Positive (+ve)
Arginine dehydrolase	Positive (+ve)
Hyalurodinase	Positive (+ve)
Lipase	Positive (+ve)

Antibiotic Sensitivity Test (Kirby- Bauer Method)

Antibiotics Used	Zone Of Inhibition (mm)	Interpretation
Ciprofloxacin (CIP) (30µg)		
Tobramycin (TOB) (10µg)		
Cotrimoxazole (COT) (25µg)		
Penicillin (P) (10µg)		
Imipenem (IPM) (10µg)		
Cefoxitin (CX) (30µg)		
Amikacin (AK) (30µg),		
Netilmicin sulphate (NET) (30 µg)		
Oxacillin (OX) (1µg),		
Erythromycin (E) (15µg),		
Clindamycin (CD) (2µg)		
Azithromycin (AZM) (15µg)		
Gentamycin (GEN) (10µg)		
Tecoplanin (TEI) (30µg)		
Linezoid (LZ) (30µg)		
Tetracycline (TE) (30µg)		
Vancomycin (VA)(30µg)		
Norfloxacin (NX) (10µg)		
Nitrofurantoin (NIT) (300µg)		
Olfloxacin (OF) (5µg)		
Chloramphenicol (C) (30µg)		

Table 5. CLSI-recommended reference MIC and zone diameter breakpoints for S. aureus[4]

Antimicrobial agent	Disc content (μg) *	Zone diameter breakpoints (nearest mm)*			MIC standard (μg/mL)*		
		S	I	R	S	I	R
Methicillin	5	≥ 14	10-13	≤ 9	≤ 8	—	≥ 16
Oxacillin	1	≥ 13	11-12	≤ 10	≤ 2	—	≥ 4
Cefoxitin	30	≥ 22	—	≤ 21	≤ 4	—	≥ 8
Vancomycin	—	—	—	—	≤ 2	4-8	≥ 16
Teicoplanin	30	≥ 14	11-13	≤ 10	≤ 8	16	≥ 32
Clindamycin	2	≥ 21	15-20	≤ 14	≤ 0.5	1-2	≥ 4
Daptomycin	—	—	—	—	≤ 1	—	—
Linezolid	30	≥ 21	—	—	≤ 4	—	—
Rifampin	5	≥ 20	17-19	≤ 16	≤ 1	2	≥ 4
Quinupristin-dalfopristin	15	≥ 19	16-18	≤ 15	≤ 1	2	≥ 4
Trimethoprim-sulfamethoxazole	1.25/23.75	≥ 16	11-15	≤ 10	≤ 2/38	—	≥ 4/76

S – Susceptible; I – Intermediate resistance; R – Resistant

*Reproduced, with permission, from CLSI publication M100-S19, Performance Standards for Antimicrobial Susceptibility Testing, Table 2C. Copies of the current edition may be obtained from Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA. www.clsi.org

Table 2C. (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Zone Diameter Interpretive Criteria (nearest whole mm) S	I	R	MIC Interpretive Criteria (µg/mL) S	I	R	Comments
PENICILLINASE-STABLE PENICILLINS (Continued)									
A	Oxacillin (For *S. aureus* and *S. lugdunensis*)		–	–	–	≤2 (oxacillin)	–	≥4 (oxacillin)	For use with *S. aureus* and *S. lugdunensis*. **(12)** Oxacillin disk testing is not reliable. See cefoxitin and comment (5) for reporting oxacillin when testing cefoxitin as a surrogate agent.
		30 µg cefoxitin (surrogate test for oxacillin)	≥22	–	≤21	≤4 (cefoxitin)	–	≥8 (cefoxitin)	**(13)** Cefoxitin is tested as a surrogate for oxacillin; report oxacillin susceptible or resistant based on the cefoxitin result. See comments (5), (8), and (11).
A	Oxacillin (For CoNS except *S. lugdunensis* **and** ***S. pseudintermedius***)	–	–	–	–	≤0.25 (oxacillin)	–	≥0.5 (oxacillin)	For use with CoNS except *S. lugdunensis* **and** ***S. pseudintermedius***. **(14)** Oxacillin MIC interpretive criteria may overcall resistance for some CoNS, because some non–*S. epidermidis* strains for which the oxacillin MICs are 0.5–2 µg/mL lack *mecA*. For serious infections with CoNS other than *S. epidermidis*, testing for *mecA* or for PBP 2a or with cefoxitin disk diffusion may be appropriate for strains for which the oxacillin MICs are 0.5–2 µg/mL.
		30 µg cefoxitin (surrogate test for oxacillin)	≥25	–	≤24	–	–	–	See comments (5), (8), (11), and (13).
A	**Oxacillin** **(For *S. pseudintermedius*)**	**1 µg oxacillin**	**≥18**	–	**≤17**	**≤0.25**	–	**≥0.5**	**(15) Neither cefoxitin MIC nor cefoxitin disk tests are reliable for detecting *mecA*-mediated resistance in *S. pseudintermedius*.**
CEPHEMS (PARENTERAL)									
B	Ceftaroline	30 µg	≥24	21–23	≤20	≤1	2	≥4	**(16)** For **reporting against** *S. aureus* only, including MRSA. **(17)** Interpretive criteria are based on a dosage regimen of 600 mg every 12 h.

Table 2C. (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Zone Diameter Interpretive Criteria (nearest whole mm) S	I	R	MIC Interpretive Criteria (µg/mL) S	I	R	Comments
GLYCOPEPTIDES **(18)** For *S. aureus*, vancomycin-suscept ble isolates may become vancomycin intermediate during the course of prolonged therapy.									
B	Vancomycin **(For *S. aureus*)**	–	–	–	–	≤2	4–8	≥16	For use with *S. aureus*. **(19)** MIC tests should be performed to determine the susceptibility of all isolates of staphylococci to vancomycin. The disk test does not differentiate vancomycin-susceptible isolates of *S. aureus* from vancomycin-intermediate isolates, nor does the test differentiate among vancomycin-susceptible, -intermediate, and -resistant isolates of CoNS, all of which give similar size zones of inhibition. **(20)** Send any *S. aureus* for which the vancomycin is ≥8 µg/mL to a reference laboratory. See Appendix A. Also refer to Table 3F for *S. aureus*, Subchapter 3.13.1.7 in M07-A10, and Subchapter 3.9.1.7 in M02-A12.
B	Vancomycin **(For CoNS)**	–	–	–	–	≤4	8–16	≥32	For use with CoNS. See comment (19). **(21)** Send any CoNS for which the vancomycin MIC is ≥ 32 µg/mL to a reference laboratory. See Appendix A. See also Subchapter 3.13.1.7 in M07-A10, and Subchapter 3.9.1.7 in M02-A12.
Inv.	Teicoplanin	30 µg	≥14	11–13	≤10	≤8	16	≥32	**(22)** Teicoplanin disk diffusion interpretive criteria were not reevaluated concurrent with the reevaluation of vancomycin disk diffusion interpretive criteria. Therefore, the ability of these teicoplanin interpretive criteria to differentiate teicoplanin-intermediate and teicoplanin-resistant staphylococci from teicoplanin-susceptible strains is not known.
LIPOGLYCOPEPTIDES									
C	**Oritavancin**	–	–	–	–	**≤0.12**	–	–	**See comment (16).**
C	**Telavancin**	**30 µg**	**≥15**	–	–	**≤0.12**	–	–	**See comment (16).**

Table 2C. (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Zone Diameter Interpretive Criteria (nearest whole mm)			MIC Interpretive Criteria (µg/mL)			Comments
			S	I	R	S	I	R	
LIPOPEPTIDES									
B	Daptomycin	–	–	–	–	≤1	–	–	(23) Daptomycin should not be reported for isolates from the respiratory tract.
AMINOGLYCOSIDES									
(24) For staphylococci that test susceptible, aminoglycosides are used only in combination with other active agents that test susceptible.									
C	Gentamicin	10 µg	≥15	13–14	≤12	≤4	8	≥16	
O	Amikacin	30 µg	≥17	15–16	≤14	≤16	32	≥64	
O	Kanamycin	30 µg	≥18	14–17	≤13	≤16	32	≥64	
O	Netilmicin	30 µg	≥15	13–14	≤12	≤8	16	≥32	
O	Tobramycin	10 µg	≥15	13–14	≤12	≤4	8	≥16	
MACROLIDES									
(25) Not routinely reported on organisms isolated from the urinary tract.									
A	Azithromycin or	15 µg	≥18	14–17	≤13	≤2	4	≥8	
A	clarithromycin or	15 µg	≥18	14–17	≤13	≤2	4	≥8	
A	erythromycin	15 µg	≥23	14–22	≤13	≤0.5	1–4	≥8	
O	Telithromycin	15 µg	≥22	19–21	≤18	≤1	2	≥4	
O	Dirithromycin	15 µg	≥19	16–18	≤16	≤2	4	≥8	
TETRACYCLINES									
(26) Organisms that are susceptible to tetracycline are also considered susceptible to doxycycline and minocycline. However, some organisms that are intermediate or resistant to tetracycline may be susceptible to doxycycline, minocycline, or both.									
B	Tetracycline	30 µg	≥19	15–18	≤14	≤4	8	≥16	
B	Doxycycline	30 µg	≥16	13–15	≤12	≤4	8	≥16	
B	Minocycline	30 µg	≥19	15–18	≤14	≤4	8	≥16	See comment (25).
FLUOROQUINOLONES									
(27) *Staphylococcus* spp. may develop resistance during prolonged therapy with quinolones. Therefore, isolates that are initially susceptible may become resistant within 3 to 4 days after initiation of therapy. Testing of repeat isolates may be warranted.									
C	Ciprofloxacin or	5 µg	≥21	16–20	≤15	≤1	2	≥4	
C	levofloxacin	5 µg	≥19	16–18	≤15	≤1	2	≥4	
C	Moxifloxacin	5 µg	≥24	21–23	≤20	≤0.5	1	≥2	
U	Norfloxacin	10 µg	≥17	13–16	≤12	≤4	8	≥16	
O	Enoxacin	10 µg	≥18	15–17	≤14	≤2	4	≥8	(28) FDA approved for *S. saprophyticus* and *S. epidermidis* (but not for *S. aureus*).
O	Gatifloxacin	5 µg	≥23	20–22	≤19	≤0.5	1	≥2	
O	Grepafloxacin	5 µg	≥18	15–17	≤14	≤1	2	≥4	
O	Lomefloxacin	10 µg	≥22	19–21	≤18	≤2	4	≥8	
O	Ofloxacin	5 µg	≥18	15–17	≤14	≤1	2	≥4	

Table 2C. (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Zone Diameter Interpretive Criteria (nearest whole mm)			MIC Interpretive Criteria (µg/mL)			Comments
			S	I	R	S	I	R	
FLUOROQUINOLONES (Continued)									
O	Sparfloxacin	5 µg	≥19	16–18	≤15	≤0.5	1	≥2	
Inv.	Fleroxacin	5 µg	≥19	16–18	≤15	≤2	4	≥8	
NITROFURANTOINS									
U	Nitrofurantoin	300 µg	≥17	15–16	≤14	≤32	64	≥128	
LINCOSAMIDES									
A	Clindamycin	2 µg	≥21	15–20	≤14	≤0.5	1–2	≥4	(29) Inducible clindamycin resistance can be detected by disk diffusion using the D-zone test or by broth microdilution (see Table 3G, Subchapter 3.10.1 in M02-A12, and Subchapter 3.14.1 in M07-A10). See comment (25).
FOLATE PATHWAY INHIBITORS									
A	Trimethoprim-sulfamethoxazole	1.25/23.75 µg	≥16	11–15	≤10	≤2/38	–	≥4/76	
U	Sulfonamides	250 or 300 µg	≥17	13–16	≤12	≤256	–	≥512	(30) Sulfisoxazole can be used to represent any of the currently available sulfonamide preparations.
U	Trimethoprim	5 µg	≥16	11–15	≤10	≤8	–	≥16	
PHENICOLS									
C	Chloramphenicol	30 µg	≥18	13–17	≤12	≤8	16	≥32	See comment (25).
ANSAMYCINS									
B	Rifampin	5 µg	≥20	17–19	≤16	≤1	2	≥4	(31) *Rx:* Rifampin should not be used alone for antimicrobial therapy.
STREPTOGRAMINS									
O	Quinupristin-dalfopristin	15 µg	≥19	16–18	≤15	≤1	2	≥4	(32) For reporting against methicillin-susceptible *S. aureus*.
OXAZOLIDINONES									
B	Linezolid	30 µg	≥21	–	≤20	≤4	–	≥8	(33) When testing linezolid, disk diffusion zones should be examined using transmitted light. Organisms with resistant results by disk diffusion should be confirmed using an MIC method.
B	Tedizolid	–	–	–	–	**≤0.5**	1	**≥2**	**See comment (16).**

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; CoNS, coagulase-negative staphylococci; FDA, US Food and Drug Administration; I, intermediate; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; MRS, methicillin-resistant staphylococci; MRSA, methicillin-resistant *S. aureus*; PBP 2a, penicillin-binding protein 2a; PCR, polymerase chain reaction; QC, quality control; R, resistant; S, susceptible; UTI, urinary tract infection.

Table 3D. Test for Detection of β-Lactamase Production in *Staphylococcus* species

Test	β-Lactamase Production	
Organism Group	*S. aureus* with penicillin MICs ≤0.12 µg/mL or zones ≥29 mm[a]	*S. aureus*[a] and CoNS (including *S. lugdunensis*[b]) with penicillin MICs ≤ 0.12 µg/mL or zones ≥ 29 mm
Test Method	Disk diffusion (Penicillin zone-edge test)	Nitrocefin-based test
Medium	MHA	N/A
Antimicrobial Concentration	10 units penicillin disk	N/A
Inoculum	Standard disk diffusion procedure	Induced growth (ie, growth taken from the zone margin surrounding a penicillin or cefoxitin disk test on either MHA or a blood agar plate after 16–18 hours of incubation)
Incubation Conditions	35°C ± 2°C; ambient air	Room temperature
Incubation Length	16–18 hours	Up to 1 hour for nitrocefin-based test or follow manufacturer's directions
Results	Sharp zone edge ("cliff") = β-lactamase positive (see Figure 1 below this table). Fuzzy zone edge ("beach") = β-lactamase negative (see Figure 2 below this table).	Nitrocefin-based test: conversion from yellow to red/pink = β-lactamase positive.
Organism Group	*S. aureus* with penicillin MICs ≤0.12 µg/mL or zones ≥29 mm[a]	*S. aureus*[a] and CoNS (including *S. lugdunensis*[b]) with penicillin MICs ≤ 0.12 µg/mL or zones ≥ 29 mm
Test Method	Disk diffusion (Penicillin zone-edge test)	Nitrocefin-based test
Additional Testing and Reporting	β-lactamase-positive staphylococci are resistant to penicillin, amino-, carboxy-, and ureidopenicillins.	Nitrocefin-based tests can be used for *S. aureus*, but negative results should be confirmed with the penicillin zone-edge test before reporting penicillin as susceptible. β-lactamase-positive staphylococci are resistant to penicillin, amino-, carboxy-, and ureidopenicillins.
QC Recommendations – Routine[c]	*S. aureus* ATCC® 25923 for routine QC of penicillin disk to include examination of zone edge test (fuzzy edge = "beach")	

Table 3D. (Continued)

Test	β-Lactamase Production	
QC Recommendations – Lot/shipment[d]		*S. aureus* ATCC® 29213 – positive *S. aureus* ATCC® 25923 – negative (or see local regulations and manufacturers' recommendations)
QC Recommendations – Supplemental*	*S. aureus* ATCC® 29213 – positive penicillin zone-edge test (sharp edge = "cliff")	

Abbreviations: ATCC®, American Type Culture Collection; CoNS, coagulase-negative staphylococci; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control.

Footnotes

a. The penicillin disk diffusion zone-edge test was shown to be more sensitive than nitrocefin-based tests for detection of β-lactamase production in *S. aureus*. The penicillin zone-edge test is recommended if only one test is used for β-lactamase detection. However, some laboratories may choose to perform a nitrocefin-based test first and, if this test is positive, report the results as positive for β-lactamase (or penicillin resistant). If the nitrocefin test is negative, the penicillin zone-edge test should be performed before reporting the isolate as penicillin susceptible in cases where penicillin may be used for therapy (eg, endocarditis).

References:

Kaase M, Lenga S, Friedrich S, et al. Comparison of phenotypic methods for penicillinase detection in *Staphylococcus aureus*. *Clin Microbiol Infect*. 2008;14(6):614-616.

Gill VJ, Manning CB, Ingalls CM. Correlation of penicillin minimum inhibitory concentrations and penicillin zone edge appearance with staphylococcal beta-lactamase production. *J Clin Microbiol*. 1981;14(4):437-440.

b. For *S. lugdunensis*, tests for β-lactamase detection are not necessary because isolates producing a β-lactamase will test penicillin resistant (MIC > 0.12 µg/mL and zone diameters < 29 mm). If a laboratory is using a method other than the CLSI disk diffusion or MIC reference method and is unsure if the method can reliably detect penicillin resistance with contemporary isolates of *S. lugdunensis*, the laboratory should perform an induced nitrocefin assay or other CLSI reference method on isolates that test penicillin susceptible before reporting the isolate as penicillin susceptible.

c. QC recommendations – Routine

Test negative (susceptible) QC strain:

- With each new lot/shipment of testing materials
- Weekly if the test is performed at least once a week and criteria for converting from daily to weekly QC testing have been met (see Subchapters 4.7.2.1 and 4.7.2.2 in M02-A12 and M07-A10)
- Daily if the test is performed less than once per week and/or if criteria for converting from daily to weekly QC testing have not been met

d. QC recommendations – Lot/shipment

Test positive (resistant) QC strain at minimum with each new lot/shipment of testing materials.

Table 3E. Test for Detection of Methicillin Resistance (Oxacillin Resistance) in *Staphylococcus* species, Except *Staphylococcus pseudintermedius*

Test	Oxacillin Resistance	*mecA*-Mediated Oxacillin Resistance Using Cefoxitin		
Organism Group	*S. aureus*	*S. aureus* and *S. lugdunensis*	CoNS[a]	*S. aureus* and *S. lugdunensis*
Test Method	Agar dilution	Disk diffusion		Broth microdilution
Medium	MHA with 4% NaCl	MHA		CAMHB
Antimicrobial Concentration	6 µg/mL oxacillin	30 µg cefoxitin disk		4 µg/mL cefoxitin
Inoculum	Direct colony suspension to obtain 0.5 McFarland turbidity. Using a 1-µL loop that was dipped in the suspension, spot an area 10–15 mm in diameter. Alternatively, using a swab dipped in the suspension and expressed, spot a similar area or streak an entire quadrant.	Standard disk diffusion procedure		Standard broth microdilution procedure
Incubation Conditions	33 to 35°C; ambient air (Testing at temperatures above 35°C may not detect MRSA.)	33 to 35°C; ambient air (Testing at temperatures above 35°C may not detect MRSA.)		33 to 35°C; ambient air (Testing at temperatures above 35°C may not detect MRSA.)
Incubation Length	24 hours; read with transmitted light	16–18 hours	24 hours (may be reported after 18 hours, if resistant)	16–20 hours

Table 3E. (Continued)

Test	Oxacillin Resistance	*mecA*-Mediated Oxacillin Resistance Using Cefoxitin		
Organism Group	*S. aureus*	*S. aureus* and *S. lugdunensis*	CoNS[a]	*S. aureus* and *S. lugdunensis*
Test Method	Agar dilution	Disk diffusion		Broth microdilution
Results	Examine carefully with transmitted light for > 1 colony or light film of growth. > 1 colony = oxacillin resistant	≤ 21 mm = *mecA* positive ≥ 22 mm = *mecA* negative	≤ 24 mm = *mecA* positive ≥ 25 mm = *mecA* negative	> 4 µg/mL = *mecA* positive ≤ 4 µg/mL = *mecA* negative
Additional Testing and Reporting	Oxacillin-resistant staphylococci are resistant to all β-lactam agents; other β-lactam agents should be reported as resistant or should not be reported.	Cefoxitin is used as a surrogate for *mecA*-mediated oxacillin resistance. Isolates that test as *mecA* positive should be reported as oxacillin (not cefoxitin) resistant; other β-lactam agents, except those with anti-MRSA activity, should be reported as resistant or should not be reported.		Cefoxitin is used as a surrogate for *mecA*-mediated oxacillin resistance. Isolates that test as *mecA* positive should be reported as oxacillin (not cefoxitin) resistant; routine testing of other β-lactam agents, except those with anti-MRSA activity, is not advised. Because of the rare occurrence of oxacillin resistance mechanisms other than *mecA*, isolates that test as *mecA* negative, but for which the oxacillin MICs are resistant (MIC ≥ 4 µg/mL), should be reported as oxacillin resistant.
QC Recommend-ations – Routine[b]	*S. aureus* ATCC® 29213 – Suscept ble (with each test day)	*S. aureus* ATCC® 25923 – *mecA* negative (cefoxitin zone 23–29 mm)		*S. aureus* ATCC® 29213 – *mecA* negative (cefoxitin MIC 1–4 µg/mL)
QC Recommend-ations – Lot/shipment[c]	*S. aureus* ATCC® 43300 – Resistant	*S. aureus* ATCC® 43300 – *mecA* positive (zone ≤ 21 mm)		*S. aureus* ATCC® 43300 – *mecA* positive (MIC > 4 µg/mL)

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; CoNS, coagulase-negative staphylococci; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; MRSA, methicillin-resistant *S. aureus*; QC, quality control.

Table 3E. (Continued)

Footnotes

a. Except *S. lugdunensis*, which is included in the *S. aureus* group.

b. QC recommendations – Routine

Test negative (susceptible) QC strain:

- With each new lot/shipment of testing materials
- Weekly if the test is performed at least once a week and criteria for converting from daily to weekly QC testing have been met (see Subchapters 4.7.2.1 and 4.7.2.2 in M02-A12 and M07-A10)
- Daily if the test is performed less than once per week and/or if criteria for converting from daily to weekly QC testing have not been met

c. QC recommendations – Lot/shipment

Test positive (resistant) QC strain at minimum with each new lot/shipment of testing materials.

Table 3F. Vancomycin Agar Screen for *Staphylococcus aureus* and *Enterococcus* species

Screen Test	Vancomycin MIC ≥ 8 µg/mL	
Organism Group	*S. aureus*	*Enterococcus* spp.
Test Method	Agar dilution	Agar dilution
Medium	BHI agar	BHI[a] agar
Antimicrobial Concentration	6 µg/mL vancomycin	6 µg/mL vancomycin
Inoculum	Direct colony suspension to obtain 0.5 McFarland turbidity. Preferably, using a micropipette, spot a 10-µL drop onto agar surface. Alternatively, using a swab dipped in the suspension and the excess liquid expressed, spot an area 10–15 mm in diameter or streak a portion of the plate.	1–10 µL of a 0.5 McFarland suspension spotted onto agar surface. Alternatively, using a swab dipped in the suspension and the excess liquid expressed, spot an area 10–15 mm in diameter or streak a portion of the plate.
Incubation Conditions	35°C ± 2°C; ambient air	35°C ± 2°C; ambient air
Incubation Length	24 hours	24 hours
Results	Examine carefully with transmitted light for > 1 colony or light film of growth. > 1 colony = Presumptive reduced susceptibility to vancomycin	> 1 colony = Presumptive vancomycin resistance
Additional Testing and Reporting	Perform a vancomycin MIC using a validated MIC method to determine vancomycin MICs on *S. aureus* that grow on BHI–vancomycin screening agar. Testing on BHI–vancomycin screening agar does not reliably detect all vancomycin-intermediate *S. aureus* strains. Some strains for which the vancomycin MICs are 4 µg/mL will fail to grow.	Perform vancomycin MIC on *Enterococcus* spp. that grow on BHI–vancomycin screening agar and test for motility and pigment production to distinguish species with acquired resistance (eg, *vanA* and *vanB*) from those with intrinsic, intermediate-level resistance to vancomycin (eg, *vanC*), such as *Enterococcus gallinarum* and *Enterococcus casseliflavus*, which often grow on the vancomycin screen plate. In contrast to other enterococci, *E. casseliflavus* and *E. gallinarum* with vancomycin MICs of 8–16 µg/mL (intermediate) differ from vancomycin-resistant enterococcus for infection control purposes.
QC Recommendations – Routine[b]	*Enterococcus faecalis* ATCC® 29212 – Susceptible	*E. faecalis* ATCC® 29212 – Susceptible
QC Recommendations – Lot/shipment[c]	*E. faecalis* ATCC® 51299 – Resistant	*E. faecalis* ATCC® 51299 – Resistant

Abbreviations: ATCC®, American Type Culture Collection; BHI, Brain Heart Infusion; MIC, minimal inhibitory concentration; QC, quality control.

Table 3F. (Continued)

Footnotes

a. BHI: even though not as widely available, dextrose phosphate agar and broth have been shown in limited testing to perform comparably.

b. QC recommendations – Routine

Test negative (susceptible) QC strain:

- With each new lot/shipment of testing materials
- Weekly if the test is performed at least once a week and criteria for converting from daily to weekly QC testing have been met (see Subchapters 4.7.2.1 and 4.7.2.2 in M02-A12 and M07-A10)
- Daily if the test is performed less than once per week and/or if criteria for converting from daily to weekly QC testing have not been met

c. QC recommendations – Lot/shipment

Test positive (resistant) QC strain at minimum with each new lot/shipment of testing materials.

Table 3G. Test for Detection of Inducible Clindamycin Resistance in *Staphylococcus* species, *Streptococcus pneumoniae*, and *Streptococcus* spp. β-Hemolytic Group[a]

Test	Inducible Clindamycin Resistance			
Test Method	Disk Diffusion (D-zone test)		Broth Microdilution	
Organism Group (applies only to organisms resistant to erythromycin and susceptible or intermediate to clindamycin)	*S. aureus*, *S. lugdunensis*, and CoNS	*S. pneumoniae* and β-hemolytic *Streptococcus* spp.	*S. aureus*, *S. lugdunensis*, and CoNS[b]	*S. pneumoniae* and β-hemolytic *Streptococcus* spp.
Medium	MHA or blood agar purity plate used with MIC tests	MHA supplemented with sheep blood (5% v/v) or TSA supplemented with sheep blood (5% v/v)	CAMHB	CAMHB with LHB (2.5% to 5% v/v)
Antimicrobial Concentration	15-µg erythromycin and 2-µg clindamycin disks spaced 15–26 mm apart	15-µg erythromycin disk and 2-µg clindamycin disk spaced 12 mm apart	4 µg/mL erythromycin and 0.5 µg/mL clindamycin in same well	1 µg/mL erythromycin and 0.5 µg/mL clindamycin in same well
Inoculum	Standard disk diffusion procedure or heavily inoculated area of purity plate	Standard disk diffusion procedure	Standard broth microdilution procedure	
Incubation Conditions	35°C ± 2°C; ambient air	35°C ± 2°C; 5% CO_2	35°C ± 2°C; ambient air	
Incubation Length	16–18 hours	20–24 hours	18–24 hours	20–24 hours
Results	Flattening of the zone of inhibition adjacent to the erythromycin disk (referred to as a D-zone) = inducible clindamycin resistance. Hazy growth within the zone of inhibition around clindamycin = clindamycin resistance, even if no D-zone is apparent.		Any growth = inducible clindamycin resistance. No growth = no inducible clindamycin resistance.	

Table 3G. (Continued)

Test	Inducible Clindamycin Resistance			
Test Method	**Disk Diffusion (D-zone test)**		**Broth Microdilution**	
Organism Group (applies only to organisms resistant to erythromycin and susceptible or intermediate to clindamycin)	*S. aureus*, *S. lugdunensis*, and CoNS	*S. pneumoniae* and β-hemolytic *Streptococcus* spp.	*S. aureus*, *S. lugdunensis*, and CoNS[b]	*S. pneumoniae* and β-hemolytic *Streptococcus* spp.
Additional Testing and Reporting	Report isolates with inducible clindamycin resistance as "clindamycin resistant." The following comment may be included with the report: "This isolate is presumed to be resistant based on detection of inducible clindamycin resistance."			
QC Recommendations – Routine[b]	*S. aureus* ATCC® 25923 for routine QC of erythromycin and clindamycin disks	*S. pneumoniae* ATCC® 49619 for routine QC of erythromycin and clindamycin disks See Appendix C for use of supplemental QC strains.	*S. aureus* ATCC® BAA-976 or *S. aureus* ATCC® 29213 – no growth	*S. pneumoniae* ATCC® 49619 or *S. aureus* ATCC® BAA-976 – no growth
QC Recommendations – Lot/shipment[c]			*S. aureus* ATCC® BAA-977 – growth	
QC Recommendations – Supplemental[d]	*S. aureus* ATCC® BAA-976 (D-zone test negative) *S. aureus* ATCC® BAA-977 (D-zone test positive) Use of unsupplemented MHA is acceptable for these strains.		*S. aureus* ATCC® BAA-976 (no growth) *S. aureus* ATCC® BAA-977 (growth)	

Abbreviations: ATCC®, American Type Culture Collection; CAMHB, cation-adjusted Mueller-Hinton broth; CoNS, coagulase-negative staphylococci; LHB, lysed horse blood; MHA, Mueller-Hinton agar; MIC, minimal inhibitory concentration; QC, quality control; TSA, tryptic soy agar.

Footnotes

a. **NOTE:** Antimicrobial susceptibility testing (AST) of β-hemolytic streptococci need not be performed routinely (see comment [4] in Table 2H-1). When susceptibility testing is clinically indicated, it should include testing for inducible clindamycin resistance. In accordance with 2010 guidance from the Centers for Disease Control and Prevention, colonizing isolates of group B streptococci from penicillin-allergic pregnant women should be tested for inducible clindamycin resistance (see comment [11] in Table 2H-1).

Table 3G. (Continued)

Reference

Verani JR, McGee L, Schrag SJ; Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention. Prevention of perinatal group B streptococcal disease – revised guidelines from CDC, 2010. *MMWR Recomm Rep*. 2010;59(RR-10):1-36.

b. QC recommendations – Routine

Test negative (susceptible) QC strain:

- With each new lot/shipment of testing materials
- Weekly if the test is performed at least once a week and criteria for converting from daily to weekly QC testing have been met (see Subchapters 4.7.2.1 and 4.7.2.2 in M02-A12 and M07-A10)
- Daily if the test is performed less than once per week and/or if criteria for converting from daily to weekly QC testing have not been met

c. QC recommendations – Lot/shipment

Test positive (resistant) QC strain at minimum with each new lot/shipment of testing materials.

d. QC recommendations – Supplemental

- Supplemental QC strains can be used to assess a new test, for training personnel, and for competence assessment. It is not necessary to include supplemental QC strains in routine daily or weekly AST QC programs. See Appendix C, footnote i, which describes use of supplemental QC strains.

Table 1. Diameter of antibacterial inhibition zones for gram-positive bacteria

Bacteria	Crude venom (mg/mL)			Vancomycin 30 µg/mL (mm)
	1.0 (mm)	1.5 (mm)	2.0 (mm)	
Staphylococcus aureus ATCC 25923	8.0 ± 0.0	9.0 ± 0.0	9.0 ± 0.0	8.0 ± 0.0
MRSA ATCC 43300	7.3 ± 0.6	7.3 ± 0.6	7.7 ± 0.6	8.3 ± 0.6
Staphylococcus aureus ATCC 29213	7.0 ± 0.0	8.0 ± 0.0	8.3 ± 0.6	8.0 ± 0.6
Staphylococcus epidermidis ATCC 12228	6.7 ± 0.6	7.0 ± 0.0	7.7 ± 0.6	8.3 ± 0.0
Bacillus subtilis ATCC 6633	2.0 ± 0.0	2.0 ± 0.0	3.3 ± 0.6	7.7 ± 0.6
Bacillus cereus ATCC 10987	–	–	1.7 ± 0.6	6.7 ± 0.6

Values shown are mean ± SD (n = 3) and represent a venom inhibition zone in millimeters (mm), excluding 4 mm diameter of the well, after 24 hours of incubation at 37°C. –: no activity. The bacterial inocula per plate contained 1 x 10^8 colony forming units, which were introduced onto Mueller-Hinton agar surfaces. Holes of 4 mm were made onto the agar surface and venom was added.